Soil and plant effects on microbial community structure

Jeffrey S. Buyer, Daniel P. Roberts, and Estelle Russek-Cohen

Abstract: We investigated the effects of two different plant species (corn and soybean) and three different soil types on microbial community structure in the rhizosphere. Our working hypothesis was that the rhizosphere effect would be strongest on fast-growing aerobic heterotrophs, while there would be little or no rhizosphere effect on oligotrophic and other slow-growing microorganisms. Culturable bacteria and fungi had larger population densities in the rhizosphere than in bulk soil. Communities were characterized by soil fatty acid analysis and by substrate utilization assays for bacteria and fungi. Fatty acid analysis revealed a very strong soil effect but little plant effect on the microbial community, indicating that the overall microbial community structure was not affected by the rhizosphere. There was a strong rhizosphere effect detected by the substrate utilization assay for fast-growing aerobic heterotrophic bacterial community structure, with soil controls and rhizosphere samples clearly distinguished from each other. There was a much weaker rhizosphere effect on fungal communities than on bacterial communities as measured by the substrate utilization assays. At this coarse level of community analysis, the rhizosphere microbial community was impacted most by soil effects, and the rhizosphere only affected a small portion of the total bacteria.

Key words: rhizosphere, microbial community, fatty acid, substrate utilization.

Résumé : Nous avons évalué les impacts de deux espèces de plantes (maïs et soya) et de trois différents types de sols sur la structure de la flore microbienne dans la rhizosphère. Notre hypothèse de travail était que la rhizosphère aurait un impact plus important sur des hétérotrophes aérobes à croissance rapide, alors qu'elle n'aurait peu ou pas d'impact sur les oligotrophes et autres microorganismes à croissance lente. Les densités de populations de bactéries cultivables et de champignons étaient plus importantes dans la rhizosphère que dans le sol en vrac. Les communautés ont été caractérisées par une analyse des acides gras du sol et par des analyses de consommation de substrats, pour les bactéries et les champignons. L'analyse des acide gras a révélé un impact important du sol mais peu d'impact des plantes sur la flore microbienne, ce qui indique que la structure générale de la flore microbienne n'a pas été affectée par la rhizosphère. Les analyses de consommation de substrats ont révélé un fort impact de la rhizosphère sur la structure de la flore bactérienne composée d'aérobes hétérotrophes à croissance rapide; les témoins de sol et les échantillons de rhizosphère se démarquant nettement. La rhizosphère a eut un impact beaucoup plus faible sur la flore fongique par rapport à la flore bactérienne tel que déterminé par les analyses de consommation de substrats. Selon ce niveau d'analyse brute, la flore microbienne de la rhizosphère fut influencée principalement par le sol, et la rhizosphère n'a affecté qu'une petite fraction des bactéries totales.

Mots clés : rhizosphère, flore microbienne, acides gras, consommation de substrats.

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Introduction

The rhizosphere is generally defined as the narrow zone of soil directly adjacent to, and affected by, plant roots. The rhizosphere contains root exudates, leaked and secreted chemicals, sloughed root cells, and mucilages. Plant root exudates contain sugars, amino and organic acids, fatty acids and sterols, vitamins, nucleotides, and other compounds (Curl and Truelove 1986). This complex mixture of organic compounds provides a source of reduced carbon, nitrogen, and other nutrients for microorganisms.

A great deal of research has been performed on the populations and communities of bacteria and fungi in the rhizosphere and bulk soil. Compared with bulk soil, the rhizosphere has higher population densities of bacteria and fungi (Curl and Truelove 1986; Maloney et al. 1997; Marschner et al. 2002; Norton and Firestone 1991; Semenov et al. 1999). This is referred to as the rhizosphere effect. However, much of this research was done by plate count methods, and direct microscopic counts of microbes in soil and rhizosphere are always much higher than results from plate counts. It is now well established that only a small percentage of soil bacteria are readily culturable, and many species of bacteria are not detected by isolation plating (Rondon et al. 1999), although the use of dilute media and long incubation times may allow many species, previously believed

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J.S. Buyer[1] and D.P. Roberts. Sustainable Agricultural Systems Laboratory, USDA–ARS, Building 001 BARC-West, Beltsville, MD 20705-2350, U.S.A.
E. Russek-Cohen. Department of Animal and Avian Science, University of Maryland at College Park, College Park, MD 20742-2311, U.S.A.

[1]Corresponding author (e-mail: buyerj@ba.ars.usda.gov).

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Table 1. Least-square mean population densities for bacteria and fungi.

Week	Plant	Soil	Bacteria (log CFU/g)	Fungi (log mpn/g)
0	Control	Keyport	6.83	3.81
0	Control	Rumford	6.71	4.14
0	Control	Hatborough	6.81	4.56
4	Control	Keyport	6.60	4.34
4	Control	Rumford	6.54	3.99
4	Control	Hatborough	5.82	4.57
4	Corn	Keyport	6.89	5.04
4	Corn	Rumford	7.53	5.32
4	Corn	Hatborough	7.58	5.17
4	Soybean	Keyport	6.97	4.15
4	Soybean	Rumford	7.44	4.76
4	Soybean	Hatborough	7.57	4.97
8	Control	Keyport	6.30	3.96
8	Control	Rumford	6.34	4.07
8	Control	Hatborough	6.26	4.12
8	Corn	Keyport	7.49	5.63
8	Corn	Rumford	7.58	5.25
8	Corn	Hatborough	7.44	5.58
8	Soybean	Keyport	6.75	4.11
8	Soybean	Rumford	7.25	4.41
8	Soybean	Hatborough	7.44	4.98
Range of standard error			0.21–0.22	0.14–0.15

Note: There were six or seven replicate samples for each treatment so standard errors varied. mpn, most probable number.

Table 2. Significance testing results for bacterial and fungal counts.

	Contrast							
Group	Time	Soil	Plant	Soil at time 0	Time × Soil	Time × Plant	Plant × Soil	Time × Soil × Plant
Bacteria	S	NS	HS	NS	NS	NS	S	NS
Fungi	NS	HS	HS	S	NS	S	S	S

Note: P >F ≤ 0.001, HS (highly significant); P >F ≤ 0.05, S (significant); P >F > 0.05, NS (not significant).

unculturable, to be isolated and grown in the laboratory (Janssen et al. 2002).

Plate count methods indicate that Gram-negative bacteria are more dominant in the rhizosphere than in bulk soil, particularly *Pseudomonas* (Curl and Truelove 1986). However, given the limitations of plate count methods, these results must be viewed with some skepticism. The choice of isolation medium affects both the total population measured and the relative abundances of various taxonomic groups of microorganisms (Buyer 1995). It has been demonstrated that the supposed dominance of fluorescent pseudomonads in the rhizosphere is an artifact of the isolation media and conditions employed (Miller 1990). The use of culture-independent methods is needed to accurately characterize microbial communities in the soil and rhizosphere. Indeed, analysis of rhizosphere DNA indicates that Gram-positive bacteria may be more dominant in the rhizosphere than previously believed (Smalla et al. 2001).

Both soil and plant have been observed to affect microbial community structure. (Duineveld et al. 1998, 2001; Normander and Prosser 2000; Smalla et al. 2001; Wieland et al. 2001). We previously examined the effect of soil and seed type on microbial community structure in the spermosphere, which is the zone directly around germinating seeds (Buyer et al. 1999). The soil microbial community was analyzed 96 h after sowing the seeds. Soil type affected the microbial community structure far more than the plant species despite great differences in exudation by the different seed types. It seemed possible that 96 h was insufficient for differences in microbial communities to develop, and we therefore decided to continue this line of research by studying the influence of soil and plant species on rhizosphere communities.

We hypothesized that the rhizosphere effect would be most profound for fast-growing, heterotrophic, aerobic microorganisms that favor higher concentrations of nutrients because they could respond very quickly to the increased availability of nutrients in the rhizosphere. These copiotrophic, or "r"-selected microorganisms, have higher growth rates and growth yields at high substrate concentrations than oligotrophic, or "K"-selected microorganisms. We further hypothesized that oligotrophic, or

Table 3. Least-square mean absorbances (595 nm) of substrate utilization assay for bacteria.

			Substrate group					
Week	Plant	Soil	Poly	Carbo	Acid	Amin	Amac	Misc
0	Control	K	0.686	0.694	0.659	0.550	0.684	0.717
0	Control	R	0.513	0.500	0.425	0.350	0.392	0.446
0	Control	H	0.653	0.777	0.994	0.777	0.935	0.817
4	Control	K	0.416	0.442	0.372	0.257	0.336	0.370
4	Control	R	0.355	0.379	0.327	0.239	0.313	0.351
4	Control	H	0.427	0.499	0.424	0.268	0.400	0.409
4	Corn	K	0.737	1.041	0.803	0.448	0.714	0.813
4	Corn	R	0.907	0.902	0.934	0.410	0.787	0.639
4	Corn	H	0.631	0.536	0.609	0.359	0.491	0.476
4	Soybean	K	0.648	0.693	0.702	0.344	0.560	0.554
4	Soybean	R	0.687	0.581	0.707	0.484	0.610	0.505
4	Soybean	H	0.546	0.519	0.610	0.345	0.518	0.461
8	Control	K	0.414	0.453	0.393	0.270	0.372	0.376
8	Control	R	0.372	0.412	0.343	0.257	0.331	0.374
8	Control	H	0.398	0.415	0.358	0.247	0.328	0.350
8	Corn	K	0.674	1.026	0.728	0.335	0.622	0.639
8	Corn	R	0.971	1.121	0.844	0.481	0.910	0.788
8	Corn	H	0.811	1.153	0.863	0.479	0.763	0.780
8	Soybean	K	0.675	0.967	0.811	0.373	0.697	0.710
8	Soybean	R	0.795	0.863	0.758	0.397	0.705	0.701
8	Soybean	H	0.680	0.782	0.749	0.346	0.598	0.602
Range of standard error			0.071–0.100	0.089–0.125	0.060–0.086	0.041–0.058	0.066–0.092	0.069–0.099

Note: There were six or seven replicate samples for each treatment, and least-square means were adjusted using control well values and bacterial population densities as covariates, so standard errors varied. Poly, polymers; Carbo, carbohydrates; Acid, carboxylic acids; Amin, amines and amides; Amac, amino acids; Misc, miscellaneous; K, Keyport; R, Rumford; H, Hatborough.

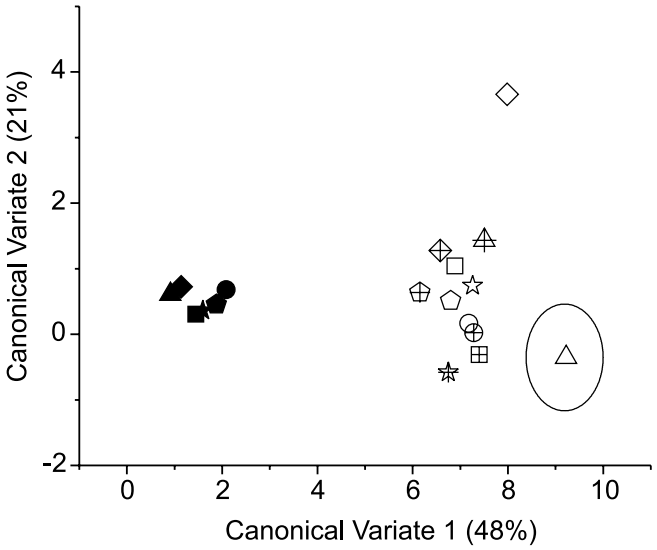
Table 4. Significance testing results for substrate utilization analysis of soil and rhizosphere bacteria.

	Contrast							
Group	Time	Soil	Plant	Soil at time 0	Time × Soil	Time × Plant	Plant × Soil	Time × Soil × Plant
Polymers	HS	NS	HS	NS	NS	NS	S	NS
Carbohydrates	HS	NS	HS	NS	NS	S	NS	NS
Carboxylic acids	HS	NS	HS	HS	NS	NS	NS	NS
Amines and amides	HS	NS	S	HS	NS	NS	NS	NS
Amino acids	HS	NS	HS	HS	NS	NS	NS	NS
Miscellaneous	HS	NS	HS	HS	NS	NS	NS	NS
Overall[a]	HS	S	HS	HS	NS	S	S	NS

Note: $P > F \leq 0.001$, HS (highly significant); $P > F \leq 0.05$, S (significant); $P > F > 0.05$, NS (not significant).
[a]Wilk's λ test.

"K"-selected microorganisms, which may represent the vast majority of soil microorganisms, and which may also be a significant percentage of so-called "unculturable" microorganisms (Watve et al. 2000), would show less response to the rhizosphere, since their growth yields and growth rates are relatively low at high concentrations of substrates (Semenov 1991). Previous studies using plate counts on rich and dilute media to measure copiotrophs and oligotrophs, respectively, partially support these hypotheses (Maloney et al. 1997; Marschner et al. 2002; Semenov et al. 1999). To test these hypotheses, we used three different methods to look at microbial community structure of the rhizosphere and soil. Fatty acid methyl ester (FAME) analysis was used to examine the overall microbial community structure, including prokaryotes and eukaryotes, independent of culturability or growth rate of the microorganisms. A substrate utilization assay (Garland and Mills 1991) was used to analyze the community structure of rapidly growing, aerobic, heterotrophic bacteria (Buyer et al. 1999), and a separate substrate utilization assay was used to examine the community structure of culturable fungi (Buyer et al. 2001). If our hypotheses were correct, we expected to see little difference between the rhizosphere and

Fig. 1. Canonical variates for bacterial substrate utilization assay. Means for each soil × plant × time combination are plotted. Ellipses represent 90% confidence intervals for the mean on the canonical variate scale and are the same size and orientation for every point within each graph. □, Keyport week 4; ○, Keyport week 8; △, Rumford week 4; ◇, Rumford week 8; ⌂, Hatborough week 4; ☆, Hatborough week 8; solid symbols, control; open symbols, corn; crossed symbols, soybean.



bulk soil using the FAME assay but substantial differences between the rhizosphere and bulk soil with the substrate utilization assays.

Materials and methods

Rhizosphere samples

Three different soil types were collected from fields in Beltsville, Md., U.S.A.: Keyport silt loam (pH 6.5, 1.75% C), Hatborough loamy sand (pH 5.9, 3.04% C), and Rumford sandy loam (pH 6.4, 0.48% C). Soils were air-dried, sieved, and stored at ambient temperature. The moisture content of the soils was restored to field capacity 1 week prior to planting. Corn (*Zea mays*) and soybean (*Glycine max* cv. Chesapeake) seeds were sown in plastic tubes (10 × 2.75 in.; 1 in. = 25.4 mm) with conical bottoms (Cone-tainers; Stuewe and Sons, Corvallis, Oreg., U.S.A.). Soil controls consisted of tubes with soil but no seeds. Plants were grown at 22°C with 14 h illumination in growth chambers.

Seven replicate tubes per soil type were taken at the time of planting for time 0 soil controls. Seven replicate Cone-tainers were harvested for each soil–plant combination, plus soil controls, at 4 and 8 weeks after sowing. Subsamples (approximately 10 g) of the soil controls were diluted 10-fold with sterile distilled water. Rhizosphere samples were harvested by emptying the Cone-tainer, cutting off the top of the plant, and adding the roots and adhering soil to 100 mL of sterile distilled water in a tared 250-mL flask. This suspension was assumed to be a 1:10 dilution, but actual sample weights were used during data workup to correct for variation in sample size. Samples were gently sonicated for 5 min and then serially diluted in sterile saline. Sample weights were used to calculate the actual dilutions. For both the controls and the rhizosphere samples, subsamples of bulk and rhizosphere soil, respectively, were stored at –20°C for fatty acid analysis. At both 4 and 8 weeks, the roots had completely penetrated all the soil in the Cone-tainers, so even the nonadhering soil was considered rhizosphere soil.

The entire experiment was performed twice. Enough soil for both experiments was collected and air-dried at one time, so the major difference between the two experiments was that the second experiment used soil that had been dry for 5 months longer before rewetting. Results were similar but not identical for the two experiments. Only data from the second experiment is presented in this paper.

Substrate utilization assays

Bacterial substrate utilization was assayed using Biolog GN plates (Biolog, Inc., Hayward, Calif., U.S.A.), as previously described (Buyer et al. 1999). A 1:10 serial dilution series in sterile saline was used to produce a 10^{-4} dilution. Each well of the plate was filled with 150 µL of the 10^{-4} dilution and incubated at 22°C. Plates were read daily, from day 3 to day 7, at a wavelength of 595 nm.

Fungal substrate utilization was assayed using Biolog SF-N plates according to a recently published procedure (Buyer et al. 2001). Briefly, a 10^{-3} dilution in 0.2% agar containing gentamycin, rifampicin, and streptomycin was used to fill plates at 100 µL per well. Plates were incubated at 22°C and read daily, from day 4 to day 10, at 650 nm.

Enumeration of bacteria and fungi

A 1:10 serial dilution series in sterile saline, done three times, was used for bacterial plate counts. Culturable bacteria were enumerated by spiral-plating (Autoplate 3000, Microbiology International, Frederick, Md., U.S.A.) dilutions onto rhizosphere isolation medium plates (Buyer 1995) in duplicate. After incubation at 22°C for 1 week, the colonies were counted using a Protocol colony counter (Microbiology International).

A most probable number assay was used to enumerate fungi (Buyer et al. 2001). Briefly, a fourfold dilution series in sterile saline was performed 11 times and eight 30-µL aliquots from each dilution were transferred to eight wells in a microplate containing 120 µL dilute rhizosphere broth (Buyer 1995) with gentamycin, rifampicin, and streptomycin. After 1 week of incubation at 22°C, wells with growth were tabulated, and the results were calculated according to the standard most probable number formula (Woomer 1994).

Fatty acid analysis

Frozen soil samples were lyophilized until dry. Fatty acids from soil lipids were directly transesterified to fatty acid methyl esters by combining 1 g of dry soil with 10 mL of 0.2 M KOH in methanol and incubating at 37°C for 1 h. Acetic acid (1 mL) and hexane (5 mL) were then added. After vortexing and centrifuging (1000 × *g*, 22°C, 10 min), the organic layer was removed. The aqueous soil suspension was extracted twice more with hexane. The hexane layers were combined and evaporated. The extract was dissolved in 0.5 mL of 1:1 hexane:methyl *t*-butyl ether and analyzed by gas chromatography. An Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, Calif., U.S.A.) was used to

Table 5. Least-square mean absorbances (650 nm) of substrate utilization assay for fungi.

			Substrate group					
Week	Plant	Soil	Poly	Carbo	Acid	Amin	Amac	Misc
0	Control	K	0.356	0.431	0.367	0.343	0.379	0.306
0	Control	R	0.561	0.609	0.471	0.314	0.459	0.501
0	Control	H	0.464	0.695	0.455	0.431	0.529	0.407
4	Control	K	0.629	0.670	0.461	0.439	0.495	0.427
4	Control	R	0.650	0.688	0.468	0.435	0.500	0.411
4	Control	H	0.709	0.768	0.502	0.522	0.565	0.384
4	Corn	K	0.699	0.784	0.537	0.631	0.620	0.466
4	Corn	R	0.705	0.738	0.460	0.464	0.565	0.373
4	Corn	H	0.555	0.597	0.423	0.479	0.481	0.317
4	Soybean	K	0.749	0.800	0.558	0.587	0.670	0.495
4	Soybean	R	0.690	0.751	0.490	0.479	0.559	0.373
4	Soybean	H	0.697	0.736	0.486	0.569	0.603	0.383
8	Control	K	0.814	0.941	0.660	0.706	0.758	0.595
8	Control	R	0.776	0.827	0.518	0.538	0.599	0.443
8	Control	H	0.714	0.902	0.563	0.607	0.628	0.432
8	Corn	K	0.717	0.766	0.514	0.616	0.636	0.438
8	Corn	R	0.766	0.926	0.553	0.650	0.712	0.397
8	Corn	H	0.760	0.848	0.531	0.683	0.627	0.429
8	Soybean	K	0.772	0.872	0.609	0.652	0.732	0.529
8	Soybean	R	0.726	0.783	0.495	0.532	0.564	0.418
8	Soybean	H	0.695	0.809	0.508	0.617	0.632	0.400
Range of standard error			0.041–0.055	0.042–0.064	0.032–0.049	0.038–0.059	0.037–0.057	0.030–0.045

Note: There were six or seven replicate samples for each treatment, and least-square means were adjusted using control well values and bacterial population densities as covariates, so standard errors varied. Poly, polymers; Carbo, carbohydrates; Acid, carboxylic acids; Amin, amines and amides; Amac, amino acids; Misc, miscellaneous; K, Keyport; R, Rumford; H, Hatborough.

Table 6. Significance testing results for substrate utilization analysis of soil and rhizosphere fungi.

	Contrast							
Group	Time	Soil	Plant	Soil at time 0	Time × Soil	Time × Plant	Plant × Soil	Time × Soil × Plant
Polymers	HS	NS	NS	HS	NS	NS	NS	NS
Carbohydrates	HS	NS	NS	HS	NS	S	S	S
Carboxylic acids	HS	S	NS	NS	NS	NS	NS	NS
Amines and amides	HS	S	NS	NS	NS	NS	NS	S
Amino acids	HS	S	NS	S	NS	S	S	S
Miscellaneous	HS	HS	NS	HS	NS	NS	NS	NS
Overall[a]	HS	HS	NS	HS	NS	NS	S	NS

Note: $P > F \leq 0.001$, HS (highly significant); $P > F \leq 0.05$, S (significant); $P > F > 0.05$, NS (not significant).
[a]Wilk's λ test.

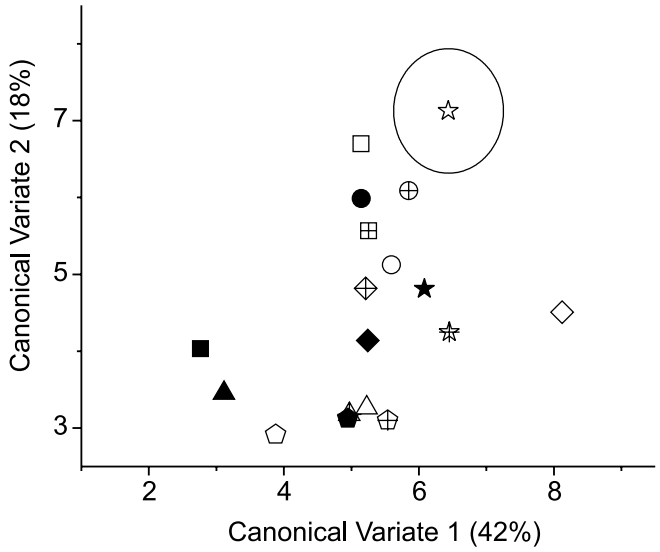
identify and quantitate fatty acid methyl esters according to the MIDI eukaryotic method (Microbial ID, Inc., Newark, Del., U.S.A.). Peak area was converted to molarity using an external standard (16:0) and assuming equimolar responses by all fatty acid methyl esters (White and Ringelberg 1998).

Data analysis

Data were analyzed using a multivariate general linear model (i.e., MANOVA) with SAS software (SAS Institute Inc., Cary, N.C., U.S.A.). At time 0, there were only controls, so the experiment was an incomplete factorial design. Therefore, customized hypothesis tests were used to test the main effects (soil at time 0, soil, plant, and time) and interactions for significance. Contrasts corresponding to main effects and interactions allowed us to include the time 0 control while still preserving the usual MANOVA sum of squares.

For the substrate utilization assay, substrates were divided into six categories (polymers, carbohydrates, carboxylic acids, amines and amides, amino acids, and miscellaneous) (Zak et al. 1994) to reduce the number of variables. The average absorbance for all wells within each category was calculated. MANOVA was conducted on the six categories using absorbance of the blank well and bacterial or fungal population density (in logarithmic units) as covariates. For

Fig. 2. Canonical variates for fungal substrate utilization assay. Means for each soil × plant × time combination are plotted. Ellipses represent 90% confidence intervals for the mean on the canonical variate scale and are the same size and orientation for every point within each graph. □, Keyport week 4; ○, Keyport week 8; △, Rumford week 4; ◇, Rumford week 8; ⌂, Hatborough week 4; ☆, Hatborough week 8; solid symbols, control; open symbols, corn; crossed symbols, soybean.



both the bacterial and fungal substrate utilization assays, the final results were very similar whether readings from 1 day were used or readings from several different days were combined to keep the average absorbance constant (Buyer et al. 2001). Only data from readings taken after 3 days of incubation for bacteria and 5 days of incubation for fungi is presented in this paper, because at these time points, no readings exceeded the absorbance limits of the microplate reader.

Fatty acids were divided into seven chemical categories (straight-chain saturated, branched-chain saturated, monounsaturated, polyunsaturated, 2-hydroxy, unsaturated branched, and oxo fatty acids) to reduce the number of variables. Peak areas were summed within each category. In addition, biomarker fatty acids were used, with the peak areas of every fatty acid contributing to a specific biomarker group summed together. The biomarker fatty acid groups were as follows: eubacteria, 15:0, 17:0 cyclo, 19:0 cyclo, 15:1 iso, 17:1 iso and anteiso; eukaryotes, polyunsaturated; Gram-positive bacteria, iso and anteiso; actinomycetes, 10 methyl 18:0; fungi, 18:2 ω6 *cis*; and protozoa, 20:3 and 20:4 (Cavigelli et al. 1995; Frostegård et al. 1993; Zelles et al. 1994, 1995). MANOVA was performed separately for chemical and biomarker groups.

We used a canonical variates analysis (Buyer et al. 1999; Seber 1984) to represent the data graphically. Since this requires a complete factorial, we ran the MANOVA only on the 4- and 8- week data. A one-way model was used in which the treatment consisted of each combination of time, soil, and plant type (or control). The canonical variates generated by this analysis identified the linear combination of variables that best separated the samples by soil and plant type at 4 and 8 weeks.

Results and discussion

Populations

Population densities of culturable bacteria and fungi are presented in Table 1, and the significance testing is shown in Table 2. There was no significant effect of soil type on bacteria, but there was a highly significant plant effect, with both soybean and corn rhizospheres having higher levels of bacteria than the soil controls. This is the expected rhizosphere effect. Fungal populations were strongly affected by soil, with Hatborough soils generally containing the largest fungal population density. There was a strong plant effect, with corn producing the highest levels of fungal population density, followed by soybean and then the soil controls. There was also a significant plant × soil interaction for both bacteria and fungi.

Substrate utilization assay

The substrate utilization assay on Biolog GN plates was used to analyze the community structure of rapidly growing, culturable, aerobic, heterotrophic bacteria. Absorbance values are presented in Table 3, and customized hypothesis testing is shown in Table 4. The rhizosphere effect is readily seen in the greater absorbances for corn and soybean than for soil controls. Both time and plant were highly significant and soil much less significant. Soil at time 0 was more significant than soil, suggesting that the portion of the microbial community measured in this assay was somewhat different in the various soils at the beginning of the experiment; however, soil effects became less important as the plants developed, and the rhizosphere effect became more significant than the soil effect. Interactions were generally less significant than main effects. The canonical variates are graphed in Fig. 1 and clearly show the effect of plants on the bacterial communities. The soil controls were clustered together and separately from all of the rhizosphere samples, indicating a strong rhizosphere effect on these bacteria. No overall difference was observed between corn and soybean rhizosphere samples.

The substrate utilization assay for fungi gave quite different results than the bacterial assay. Absorbances are presented in Table 5. There was a strong time effect, with a general increase in substrate utilization from 0 to 4 to 8 weeks. Customized hypothesis testing (Table 6) revealed that time and soil effects were far more significant than plant effects. The time effect is readily apparent in the graphed data (Fig. 2), where the separation between 4- and 8-week samples indicates a temporal effect. The soil effect is less obvious than the time effect, but the Keyport soil does tend to separate along the vertical axis from the other soils. It is clear that the rhizosphere had much less effect on fungi (Fig. 2) than it did on rapidly growing, aerobic, heterotrophic bacteria (Fig. 1).

The rhizosphere effect was most easily seen in the substrate utilization assay for fast growing, aerobic, heterotrophic bacteria. This was entirely consistent with our first hypothesis that the rhizosphere effect would be strongest for fast growing, aerobic, heterotrophic bacteria that favor high concentrations of nutrients. While these bacteria represent a very small percentage of the total community, they are ex-

Table 7. Concentration (nmol/g soil dry weight) of fatty acid chemical groups.

			Fatty acid group						
Week	Plant	Soil	Satd	Branch	Mono	Poly	Hydr2	Unsbr	Total
0	Control	K	484	284	407	91	53	99	1418
0	Control	R	235	65	118	45	10	11	485
0	Control	H	389	128	148	55	19	23	763
4	Control	K	580	301	538	132	62	94	1712
4	Control	R	249	65	202	62	7	10	596
4	Control	H	386	117	218	54	19	22	818
4	Corn	K	657	339	674	202	59	113	2047
4	Corn	R	353	78	283	146	11	15	887
4	Corn	H	530	155	412	155	25	29	1307
4	Soybean	K	626	295	636	157	59	83	1858
4	Soybean	R	291	72	255	118	8	15	759
4	Soybean	H	569	167	424	147	25	34	1368
8	Control	K	599	295	490	118	58	90	1656
8	Control	R	261	68	1798	86	6	16	617
8	Control	H	456	130	232	59	22	26	928
8	Corn	K	733	295	885	200	57	91	2262
8	Corn	R	300	40	277	98	7	10	732
8	Corn	H	526	119	363	133	22	23	1185
8	Soybean	K	642	296	645	163	65	98	1909
8	Soybean	R	261	66	194	77	9	15	622
8	Soybean	H	416	130	278	91	23	25	963
Standard error			39	14	42	19	3	6	112

Note: Numbers reported are least-square means, with seven replicate samples for each treatment. Satd, saturated; Branch, branched; Mono, monounsaturated; Poly, polyunsaturated, Hydr2, 2-hydroxy; Unsbr, unsaturated branched; K, Keyport; R, Rumford; H, Hatborough.

Table 8. Significance testing results for fatty acid analysis of rhizosphere samples.

	Contrast[a]							
Group	Time	Soil	Plant	Soil at time 0	Time × Soil	Time × Plant	Plant × Soil	Time × Soil × Plant
Saturated	NS	HS	HS	HS	NS	NS	NS	NS
Branched	S	HS	NS	HS	NS	S	NS	NS
Monounsaturated	S	HS	HS	HS	S	NS	S	NS
Polyunsaturated	NS	HS	HS	NS	NS	NS	NS	NS
2-Hydroxy	NS	HS	NS	HS	NS	NS	NS	NS
Unsaturated branched	NS	HS	NS	HS	NS	NS	NS	NS
Total	NS	HS	HS	HS	NS	NS	NS	NS
Overall[a]	HS	HS	HS	HS	NS	HS	HS	S

Note: Fatty acids were summed by chemical groups. $P > F \leq 0.001$, HS (highly significant); $P > F \leq 0.05$, S (significant); $P > F > 0.05$, NS (not significant).
[a]Wilk's λ test.

actly the bacteria expected to respond in the substrate utilization assay (Bååth et al. 1998; Buyer et al. 1999). The rhizosphere samples were clearly distinct from the soil controls. However, there was very little difference between corn and soybean rhizospheres with the substrate utilization assay, and the influence of soil type was very small. The rhizosphere effect observed here appears to be largely independent of plant species and the starting microbial community. However, the study of more plant species might lead to a different conclusion, since it may also be that the substrate utilization assay used here is capable only of detecting large changes in community structure and that subtle differences between corn and soybean rhizosphere communities could not be resolved.

The rhizosphere effect on the fungal community, as measured by the substrate utilization assay, was much weaker than that on the rapidly growing, aerobic, heterotrophic bacteria. This is consistent with literature on dilution plate counts, where the increase in fungal populations in the rhizosphere compared to bulk soil is generally less than in bacterial populations (Curl and Truelove 1986). The fungal communities in the control soils were different from each other and changed more over time than the differences between the controls and the rhizosphere communities. These

Table 9. Concentration (nmol/g soil dry weight) of fatty acid biomarkers.

			Fatty acid group[a]				
Week	Plant	Soil	Bacteria	Gram +	Protozoa	Fungi	Actinomycetes
0	Control	K	176	284	11	68	41
0	Control	R	40	65	2	43	3
0	Control	H	70	128	2	46	18
4	Control	K	179	301	15	95	36
4	Control	R	30	65	7	55	1
4	Control	H	65	117	3	47	15
4	Corn	K	203	339	21	168	40
4	Corn	R	46	78	13	133	3
4	Corn	H	92	155	24	123	18
4	Soybean	K	167	295	19	122	36
4	Soybean	R	41	72	10	108	3
4	Soybean	H	101	167	23	115	20
8	Control	K	166	295	15	88	33
8	Control	R	39	68	4	82	0
8	Control	H	81	130	9	45	16
8	Corn	K	180	295	33	151	38
8	Corn	R	30	40	18	79	0
8	Corn	H	71	119	20	105	15
8	Soybean	K	186	296	20	128	39
8	Soybean	R	40	66	8	70	1
8	Soybean	H	81	3130	11	74	17
Standard error			10	14	3	16	2

Note: Numbers are least-square means with seven replicate samples for each treatment. K, Keyport; R, Rumford; H, Hatborough.

[a]Eukaryote group is identical to the polyunsaturated group that is presented in Table 7.

Table 10. Significance testing results for fatty acid analysis.

	Contrast[a]							
Group	Time	Soil	Plant	Soil at time 0	Time × Soil	Time × Plant	Plant × Soil	Time × Soil × Plant
Eubacteria	NS	HS	NS	HS	NS	NS	NS	NS
Gram +	S	HS	NS	HS	NS	S	NS	NS
Actinomycete	S	HS	S	HS	NS	NS	NS	NS
Fungi	S	HS	HS	NS	NS	NS	NS	NS
Protozoa	NS	HS	HS	S	NS	S	NS	NS
Overall[a]	HS	HS	HS	HS	NS	HS	NS	S

Note: Fatty acids were summed by biomarker groups. $P > F \leq 0.001$, HS (highly significant); $P > F \leq 0.05$, S (significant); $P > F > 0.05$, NS (not significant).

[a]Wilk's λ test.

results are limited to fungi that can grow in this assay, which excludes a number of important taxonomic groups (Smit et al. 1999).

Fatty acid analysis

Fatty acid concentrations, summed by chemical group, are presented in Table 7. A strong soil effect is readily apparent, with the Rumford soil having much lower concentrations of fatty acids than the other two soils. This may be due to the lower carbon content of the Rumford soil. The results for the customized hypothesis testing are summarized in Table 8. In general, soil had the greatest effect on soil fatty acids, while the time and plant effects were weaker. Both soil and soil at time 0 were highly significant in affecting the groups of fatty acids, suggesting that the different soil types each started out with different microbial communities that remained different throughout the experiment. Interactions were generally much less significant than the main effects.

The results for the biomarker groups are given in Tables 9 and 10. Results are generally similar to those found for the chemical groups, with soil effects on fatty acids grouped by biomarker being generally more significant than time or plant effects. Soil at time 0 had more influence on bacteria, Gram-positive bacteria, and actinomycetes than on eukaryotes, fungi, and protozoans, suggesting that the different soil types had greater differences in prokaryotes than eukaryotes at the beginning of each experiment. The eukaryote and fungi biomarkers were strongly affected by plant, while the protozoa

Fig. 3. Canonical variates for fatty acid chemical groups. Means for each soil × plant × time combination are plotted. Ellipses represent 90% confidence intervals for the mean on the canonical variate scale and are the same size and orientation for every point within each graph. □, Keyport week 4; ○, Keyport week 8; △, Rumford week 4; ◇, Rumford week 8; ⌂, Hatborough week 4; ☆, Hatborough week 8; solid symbols, control; open symbols, corn; crossed symbols, soybean.

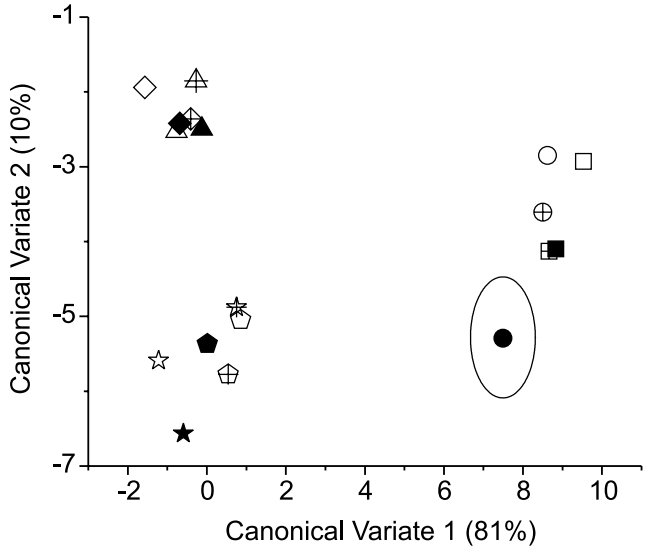
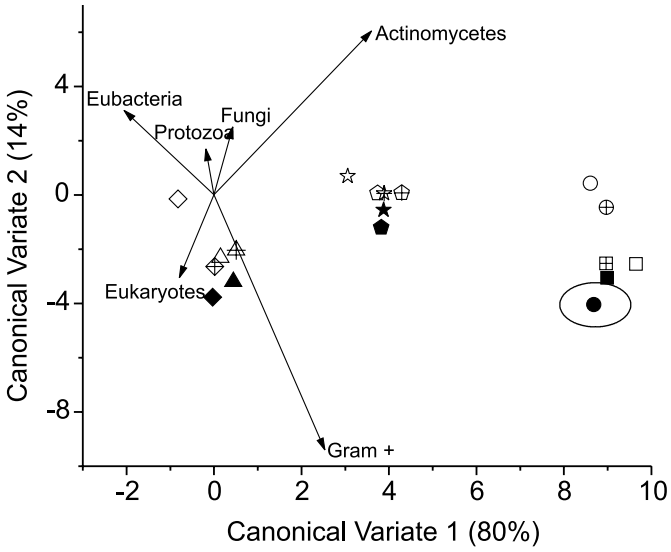


Fig. 4. Canonical variates for fatty acid biomarker groups. Means for each soil × plant × time combination are plotted. Ellipses represent 90% confidence intervals for the mean on the canonical variate scale and are the same size and orientation for every point within each graph. Vectors represent standardized canonical coefficients and indicate the relative contribution of each biomarker group to each canonical variate. □, Keyport week 4; ○, Keyport week 8; △, Rumford week 4; ◇, Rumford week 8; ⌂, Hatborough week 4; ☆, Hatborough week 8; solid symbols, control; open symbols, corn; crossed symbols, soybean.



biomarker was most strongly affected by plant and soil. The fungal biomarker fatty acid 18:2 ω6c occurs in almost all eukaryotes, including plants (Lechevalier 1988), so one possible explanation is that plant root fragments rather than fungi were detected in the fatty acid analysis of rhizosphere samples.

The canonical variates analysis for fatty acid chemical categories is graphed in Fig. 3. The three soil types remained quite distinct from each other, with little plant or temporal effect observed. However, the horizontal axis explains 81% of the variance, and the vertical axis only explains 10% of the variance, so separations along the horizontal axis are more significant than separations along the vertical axis. Therefore, the biggest difference in microbial community structure occurred between the Keyport soil samples and the other two soil types, which may be explained by the different soil textures (Gelsomino et al. 1999). The only case where corn and soybean rhizospheres had different communities from each other was with the Keyport soil at week 4.

The fatty acid biomarkers are graphed in Fig. 4. The vectors graphically represent the standardized canonical coefficients, with the length and direction of each vector indicating its relative contribution to each axis. The patterns were similar to the chemical categories, with all samples clustering by soil. The actinomycetes and Gram-positive bacteria were greatest in the Keyport soil, but in this case, the eubacteria biomarker had the largest negative effect along the horizontal axis.

Fatty acid analysis was used to look at the overall microbial community structure. Each soil started out with a different microbial community structure that was maintained through 8 weeks of plant root growth and deposition of reduced carbon and other nutrients. The rhizosphere effect on microbial community structure was quite weak compared with the soil effect. This is consistent with our second hypothesis, which held that the rhizosphere effect for slow-growing heterotrophs and oligotrophs would be very small. These organisms are believed to dominate bulk soil, so the small percentage of microorganisms that did respond strongly to the rhizosphere appeared to be insufficient to change the community structure as measured by fatty acid analysis. It is clear that the rhizosphere affects only a small fraction of the soil microbial community, and the increase in population of that small fraction is insufficient to significantly change the community structure.

In this paper, we found that soil type generally had far more influence on overall microbial community structure than plant roots, although the fast-growing, aerobic, heterotrophic bacteria that responded to the substrate utilization assay were strongly affected by the rhizosphere. Other bacteria and eukaryotes did not respond strongly to plant roots. However, since we do not know how metabolically active the oligotrophic bacteria and other slow-growing soil microorganisms are, it is difficult to use these results to make any predictions about the impact of the rhizosphere effect on ecosystem function.

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